Article Summary Line: In one of the areas of first expansion of the epidemic in Italy IgG seroprevalence for SARS-CoV-2 increases with age, possibly suggesting age related differences in susceptibility to infection.

Running Title: Seroprevalence screening in Castiglione D'Adda.

Keywords: mass screening, seroprevalence, SARS-CoV-2, COVID-19

Title: Seroprevalence of SARS-CoV-2 IgG significantly varies with age: results from a mass population screening (SARS-2-SCREEN-CdA)

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Abstract – 48 words

Castiglione D'Adda is one of the towns earlier and more severely affected by the SARS-CoV-2 epidemic in Lombardy. In a mass screening involving 4174 out of about 4550 total inhabits, significant age-related differences in anti SARS-CoV-2 IgG seroprevalence were found, with the lowest prevalence in the youngest inhabitants.

Text – 691 words

Background and Methods

Coronavirus Induced Disease 2019 (COVID-19) is caused by a novel *betacoronavirus* which was first identified in China and denominated SARS-CoV-2 (Severe Acute Respiratory Sindrome Coronavirus 2) [1]. Italy was the first European country that suffered a wide spread of the infection, which caused hundreds of thousands of cases [2].

The municipality of Castiglione d'Adda, a town of about 4550 inhabitants, has been heavily affected by SARS-CoV-2 infection since the earliest stages of the epidemic and subjected to movement restrictions since February 23rd, 2020. As of June 21, 2020, 184 confirmed cases of COVID-19 were reported, the large majority of which requiring hospitalization, accounting for about 4% of the total population. At the same time, 76 deaths were officially attributed to COVID-19, with an overall population mortality of 1.7%. During the epidemic, testing was restricted to severely symptomatic cases. Consequently, the true extent of the SARS-CoV-2 infection remains unknown. In this study, the entire population of Castiglione D'Adda was invited to perform a lateral-flow immunocromatographic tests on capillary blood (Prima Lab, Switzerland). Moreover, a random sample of 562 subjects (stratified per sex and age) and all subjects tested positive to the rapid test were invited to undergo confirmatory tests by chemiluminescent method on venipuncture drawn blood (CLIA, IgG anti-SARS-CoV-2, Abbott, USA) and SARS-CoV-2 PCR on NPS [3].

The analysis of IgG prevalence in the different age groups was performed by logistic regression models with response variable equal to 1 for positive IgG results, and 0 for negative IgG results. Age and gender were included as independent variables. Results were reported in terms of estimated probabilities of being positive to IgG test as a function of age, with respective 95% confidence intervals.

Results and Discussion

The analysis was based on 509 subjects of the random sample with available results. The overall seroprevalence found in the tested sample was 22.6% (95% confidence interval 17.2- 29.1). A significant effect of age was found ($p<0.0001$) while no significant association emerged between IgG results and gender ($p=0.2560$). The possible existence of a non-linear effect of age was tested by including spline polynomials, without significant results ($p=0.9078$). Furthermore, an age/gender interaction effect did not result significant ($p=0.5199$). Estimates of probabilities of being positive to IgG test, from a model including only age as independent variable, are reported in Fig.1 and Tab. 1.

Since the early phases of the pandemic, advanced age was identified as an independent predictor for severe disease and worse outcomes [4]. Beside this, it remains unclear if the limited number of cases reported in children [5] is due to a milder course of disease, with a larger percentage of asymptomatic cases, or to a lower susceptibility to infection, as our results seem to suggest. Different ACE2 expression according to age have been postulated to explain clinical expression and susceptibility to the infection. In particular, a higher expression of ACE2 in lung tissues in advanced age groups had been speculated [6, 7]. Moreover, a variable susceptibility to other coronavirus such as HCoV-NL63, which also use ACE2 as cell receptor in humans, in different age groups, has been also reported in different age groups [8].

Another possible explanation may be that an asymptomatic/pauci-symptomatic infection, more common in younger subjects, could elicit a less marked, or transient, antibody response, as already found in the closely related Middle East Respiratory Syndrome Coronavirus (MERS-CoV) [9].

A possible confounding factor in our findings could be related to social distancing measures: schools of any grade were among the first institutions to be closed in Italy, starting from the 5th of March. This could have led to a lower exposure to the infection in children in pre-scholar and scholar age groups.

In conclusion, our findings suggest that IgG seroprevalence for SARS-CoV-2 increases with increasing age and these data suggest a lower susceptibility to infection in the lower age groups. These findings have important implications in epidemiology and public health, particularly in designing future population screenings, and could be an important contribution in the re-opening process, especially considered that more than three-fourths of the population could be still susceptible to SARS-CoV-2 infection, even in an area of initially unrestricted viral circulation.

Conflict of interests

The authors declare no conflicts of interest.

Acknowledgments

MG, GP, FC, DB, AG, RR and EB defined the study protocol. GP drafted a first version of the manuscript, which was then revised and integrated by MG, EB, AG, CEG and SC. EB, PB and GM analyzed the data. All authors approved the final version of the manuscript.

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The study was approved by University of Milan's Ethical Committee.

Author Bio

Gabriele Pagani is an Infectious Diseases resident at Luigi Sacco Hospital (Milano, Italy), currently ending his last year of residency. His primary interests are tropical and traveler diseases and anthropo-zoonoses, particularly in One Health and Global Health perspectives.

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160 **Tab.1 Estimates of the probability of IgG positivity for age interval of five years**

161 C.I. = confidence interval

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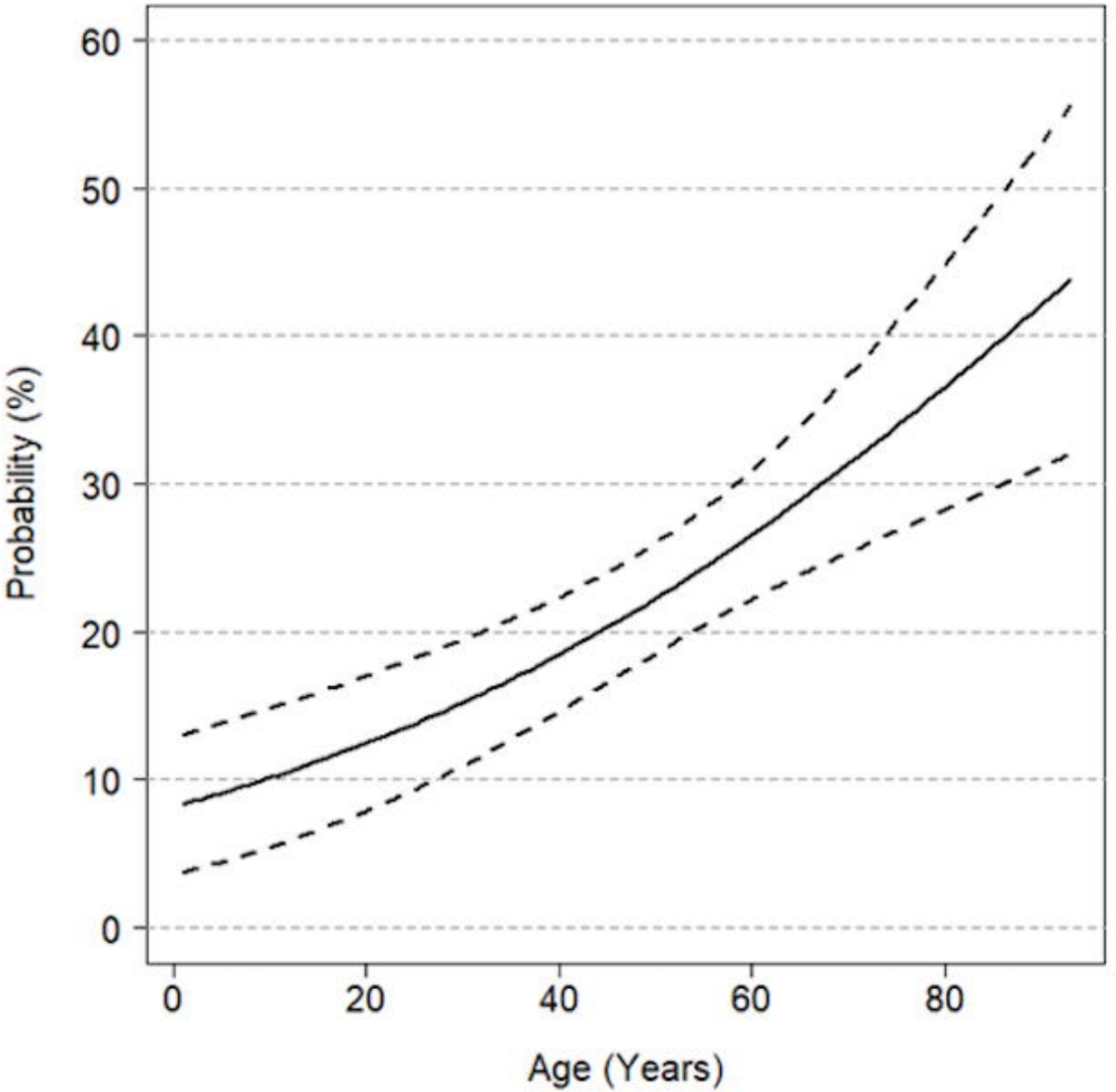
163 **Fig.1: Estimated probability of IgG positivity as a function of age**

164 Solid line: estimates, dashed lines: 95% confidence intervals.

AGE	PROBABILITY (95% C.I.)	AGE	PROBABILITY (95% C.I.)
5	9.1% (4.4%, 13.8%)	**50**	22.3% (18.5%, 26.0%)
10	10.1% (5.4%, 14.8%)	**55**	24.3% (20.4%, 28.3%)
15	11.2% (6.6%, 15.9%)	**60**	26.6% (22.2%, 30.9%)
20	12.5% (7.9%, 17.0%)	**65**	28.9% (23.8%, 33.9%)
25	13.8% (9.3%, 18.2%)	**70**	31.3% (25.4%, 37.3%)
30	15.2% (11.0%, 19.5%)	**75**	33.9% (26.8%, 41.0%)
35	16.8% (12.7%, 20.9%)	**80**	36.6% (28.3%, 44.9%)
40	18.5% (14.6%, 22.4%)	**85**	39.3% (29.7%, 48.9%)
45	20.3% (16.6%, 24.0%)	**90**	42.1% (31.1%, 53.1%)


60
50
40
30
20
10
0
Probability (%)
0
20
40
60
80
Age (Years)